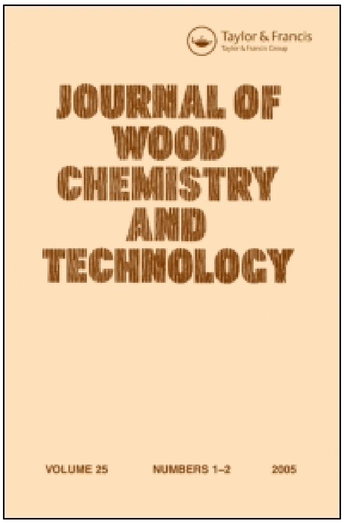

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Enzyme Treatments of the Dissolved and Colloidal Substances Present in Mill White Water and the Effects on the Resulting Paper Properties

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ENZYME TREATMENTS OF THE DISSOLVED AND COLLOIDAL SUBSTANCES PRESENT IN MILL WHITE WATER AND THE EFFECTS ON THE RESULTING PAPER PROPERTIES

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ABSTRACT

The composition of the dissolved and colloidal fractions of a "model" white water prepared from a spruce-pine-fir/hemlock thermomechanical pulp was determined. The impact of these fractions on paper properties was assessed and the ability of enzymes to degrade the different components was investigated. The colloidal particles in the white water had an average size of 0.5 μm and a size range from 0.1 μm to 2 μm. Lignins, resin and fatty acids, and esterified extractives, such as sterol esters and triglycerides, were the main constituents of the colloidal particles, while the lignans and neutral polysaccharides were predominantly dissolved in the white water. Reductions in paper strength were mainly caused by the dissolved substances, whereas the colloidal substances were

primarily responsible for the reduction in paper porosity and optical properties. Added laccases were able to degrade most of the extractives while lipases specifically hydrolyzed esterified extractives present in the colloidal fraction.

INTRODUCTION

Pulp and paper mills are being encouraged to minimize both water usage and effluent discharge. However, as a consequence of water closure, much of the contaminated water is recycled and more dissolved and colloidal substances (DCS) accumulate in the mill process water. Past research has shown[1,2] that these DCS substances can lead to problems in the papermaking process and in the quality of the resulting paper products. The effect that each of the DCS components has on paper properties is not yet clearly defined. During our previous work,[3] it was recognized that both the chemical nature of the components and their physical form (dissolved or colloidal) determine their effects on paper properties. In this study, a 0.22 μm membrane filter was used to separate newsprint mill white water colloidal particles from the dissolved substances. The chemical and physical constitutions of these two fractions were then determined. Both the original white water and white water containing only the dissolved substances, obtained after membrane filtration, were used to prepare handsheets. The physical properties of these two types of handsheets were tested and compared to the handsheets made using distilled water (DW). In this manner, the separate effects of the dissolved and colloidal fractions in white water on the paper properties were determined.

The use of enzyme systems to remove detrimental DCS substances present in various types of white water has been pursued by several research groups in the past.[4-6] Most researchers have explored the use of hydrolytic enzymes such as lipases, esterases, cellulases and pectinases which are able to degrade pitch components and destabilize the colloidal particles. In our earlier work,[7,8] we found that the fungus *Trametes versicolor* and the fungal culture filtrate (FCF) obtained from this organism, were able to efficiently degrade the dissolved and colloidal substances present in the process waters of a newsprint mill. The removal of lignans and triglycerides indicated the presence of oxidative enzymes and hydrolases in addition to the expected cellulytic enzymes in the fungal culture filtrates.[7] In order to obtain a better understanding of the impact of the FCF on the process waters, we treated the colloid containing and colloid-free white waters with four commercial enzymes, a laccase, a lipase (Resinase A 2X), a cellulase and a mannanase.

MATERIALS AND METHODS

Preparation of Recycled White Water

Thermomechanical pulp (TMP) from a mixed softwood furnish (spruce/pine/fir and hemlock) was collected from the discharge of a secondary refiner at Howe Sound Pulp and Paper Ltd., Port Mellon, BC, Canada. A model recycled white water was prepared by washing the TMP with deionized water (DW) in a batch process. In each batch, 3.6 kg of pulp was washed with 180 L DW water at 60°C for 20 min, with stirring. The pulp suspension was dewatered to about 45% consistency using a screw press and the water was collected as white water. The collected white water was used to wash a fresh batch of TMP in the same manner, in order to obtain the recycled white water (WW).

Membrane Filtration of White Water

White water was filtered through a 0.45 μm membrane filter (HAWP, Millipore) and then a 0.22 μm membrane filter (GSWP, Millipore). The final filtrates were collected as filtered white water (FWW).

Particle Size Determination

Particle size distribution was determined using Zetasizer 3 Particle electrophoresis and a Multi Angle Particle Size analyzer (Manern, Ontario), using a 5mW Helium Neon laser optical unit. The data were analyzed on a Zenith Data System using Manern application software.

White Water Chemical Analysis

The white water was extracted using methyl *t*-butyl ether (MTBE) to separate the lipophilic extractives from the highly water solubles, as described by Örså and Holmbom.[9] Total dissolved and colloidal substances (TDCS) were measured as the dry weight obtained by oven-drying a known volume of water sample at 105±3°C to constant weight. The carbohydrates present in freeze dried white water samples were measured by HPLC using the method of de Jong, et al..[10] The lignin content was determined as acid-insoluble lignin of the freeze-dried extractive free white water sample (Tappi standard method T222). The extractive groups, resin and fatty acids, lignans, steryl esters and triglycerides were measured by gas chromatography according to Örså and Holmbom.[9] Uronic acids were analyzed on CarboPac PA-1 column using a Dionex DX-500 HPLC system (Dionex, Sunnyvale, CA), controlled by Peaknet 4.10 software. The acid sugars were eluted using 10% NaOH (1M), 15% sodium acetate (1M) and 75% nanopure water. A dionex ED40 electrochemical detector (gold electrode) was used to monitor the uronic acids.

Handsheet Preparation

Handsheets were prepared according to CPPA Standard procedure C4 with modification to ensure constant fines content. As the normal procedure for maintaining fines requires recirculation of the handsheet machine water, which in turn would result in removal of the colloidal material from the white water through filtration in the first few fines-poor handsheets, the following procedure was used. The pulp slurry was enriched with fines from the original pulp to compensate for fines lost during handsheet forming. The handsheets were then prepared in one pass using the appropriate white water for dilution. Sufficient handsheets were prepared to provide enough material for a minimum of ten samples for each property test.

Handsheet Properties

The Scott bond was measured according to Tappi routine control method RC-308 using the Scott model B tester. All other handsheet physical properties were measured following CPPA standard procedures.

Enzyme treatment

The enzymes used in this study were a lipase (Resinase A 2X), a cellulase (Celluclast 1.5L) and a mannanase (Mannanase J) provided by Novo Nordisk BioChem Ltd. plus a laccase obtained from VTT Biotechnology and Food Research. All of the enzymes were in a liquid preparation. The enzyme treatment of WW and FWW was carried out in 500 mL Pyrex flasks each containing 120 mL of water sample and the appropriate mount of enzyme at 45°C for 3 h. The enzyme dosages used in the white water treatment (based on a volume of 120 mL of white water) were 16 IU for laccase, 40 IU (CMC) for cellulase, 50 MNU for mannanase and 5 IU for lipase.

RESULTS AND DISCUSSION

The Nature and Chemical Constitution of the White Water Dissolved and Colloidal Fractions

It is well known that the organic substances in the process waters of newsprint mills are present in both dissolved and colloidal forms. We have previously shown that specific organic substances in the process waters of newsprint mills have markedly different impacts on paper quality and papermachine runnability.[3] Moreover, our results indicated that the impact was highly dependent upon whether the organic substances were present in the colloidal or dissolved form.

In order to obtain a better understanding of the relationship between the colloidal and dissolved nature of the organic compounds on paper properties, it was necessary to separate the dissolved and colloidal fractions in the model white water (WW). Analysis of the size of the colloidal particles in this white water indicated that the majority of the particles were in the 250 to 1000 nm range with very few particles less than 220 nm in size (Figure 1). Thus it was possible to separate the colloidal organics from the dissolved fraction by filtration through a 0.22 μm membrane filter.

The white water (WW) and membrane filtered white water (FWW) were analyzed for total dissolved and colloidal organic substances (TDCS), and for various classes of components. As can be seen in Table 1, carbohydrates formed the majority of the TDCS together with a significant amount of lignin and

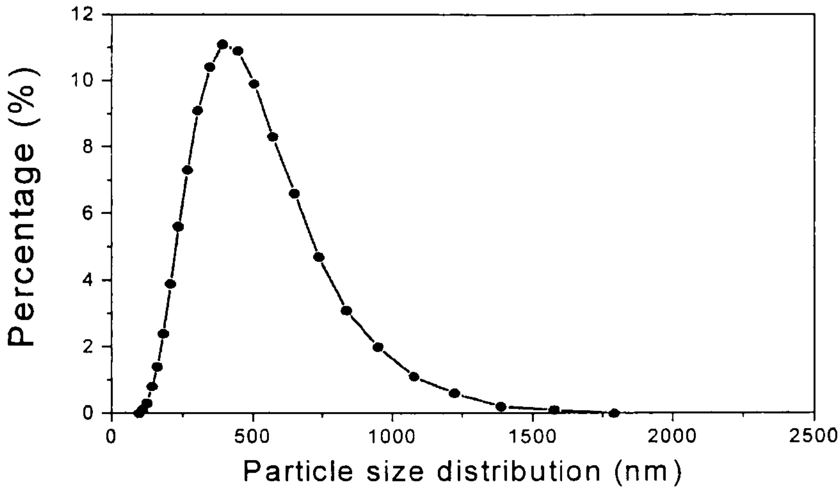

FIGURE 1. Size Distribution of White Water Colloidal Particles

TABLE 1.

The Amount (mg/L) of the Major DCS Components Present in the White Water (WW) and Membrane Filtered White Water (FWW) Samples

mg/L	White water (WW)	Filtered white water (FWW)
TDCS	2200 ± 80	1900 ± 55
Neutral sugar	1200 ± 60	1190 ± 90
Lignin	460 ± 10	600 ± 10
Extractives *		
RFA	33 ± 1	16 ± 1.4
Li&S	171 ± 2	167 ± 6.2
SE	25 ± 0.3	18 ± 3.5
TG	11 ± 4	0
Uronic acids		
Glu.U	4 ± 0.5	7 ± 0.5
Gal.U	3.8 ± 1	0.3 ± 0.2

*RFA: resin and fatty acids; Li & S: lignans and sterols; SE: steryl esters; TG: triglycerides; Gal.U: Galacturonic acid; Glu.U: Gluconic acid

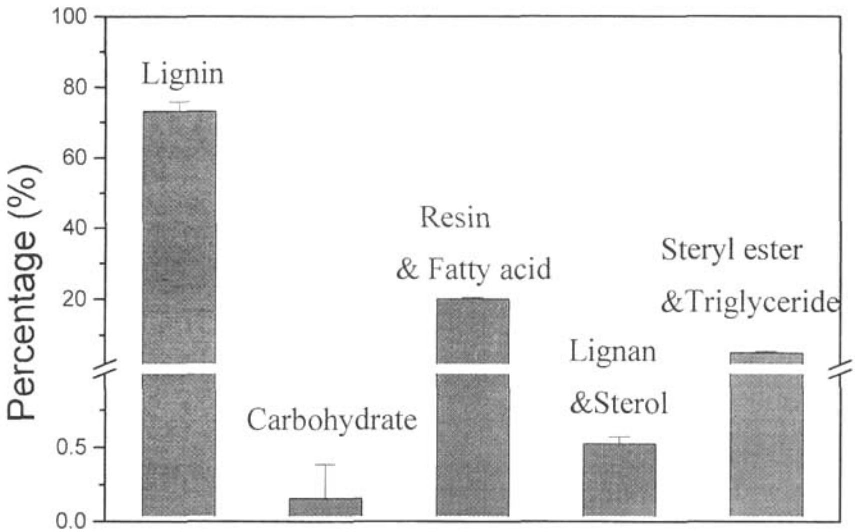

FIGURE 2. The Percentage of Each DCS Groups Present in White Water Colloidal Fraction

extractives. Filtration removed very little of the neutral polysaccharides but nearly all of the galactouronic acids and 60% of the lignin. Detailed gas chromatographic analysis of the extractives in the solution phase before and after filtration indicated that about half of the lipophilic extractives, resin and fatty acids (RFA), steryl esters (SE) and triglycerides (TG), were removed with the colloidal fraction whereas a significant amount of hydrophilic extractives, lignans and sterols (Li&S), were in solution.

By difference, we were able to calculate the percentage composition of the colloidal fraction. As shown in Figure 2, the colloidal fraction consists mainly of lignin with smaller amounts of resin and fatty acids, and esters. The content of neutral carbohydrates was very small.

The Effects of Dissolved and Colloidal Substances on Paper Properties

Relatively low concentrations of dissolved and colloidal substances in the white water of the papermachine can have a large effect on the quality of paper produced.[3] In order to develop methods for reducing the problems encountered with the DCS in papermaking, it is important to understand not only the effects of each specific class of organic substance, but to also understand the impact of the physical form of the substance. In order to develop an understanding of the impact of the physical form of the organic components, it was necessary to prepare, through filtration, a large amount of white water from which the colloidal substances had been removed. This colloid-free white water (FWW), the original white water (WW) and deionized water (DW) were used to prepare handsheets. The pulp for the handsheets was a screened thermomechanical pulp made from the same type of softwood furnish as was used by Howe Sound Pulp and Paper Ltd. during white water collection.

The density of handsheets made using WW and FWW were 0.283 g/cm^3 and 0.276 g/cm^3 respectively. These values are significantly lower than the value of 0.316 g/cm^3 obtained for the density of handsheets prepared using deionized water. This reduction in sheet consolidation is likely caused by the presence of dissolved resin and fatty acids. The reduced sheet consolidation is reflected in reduced internal bonding (Scott bond) and tensile strength (Figure 3). The presence of the colloidal fraction had no significant effect on the physical properties.

In contrast, the presence of the colloidal fraction in the white water had a marked effect on sheet porosity, moisture absorption and brightness (Figure 4). Handsheets made using the white water that contained only the dissolved components, showed a more rapid absorption of moisture and a higher porosity when compared to handsheets made with deionized water. This is to be expected

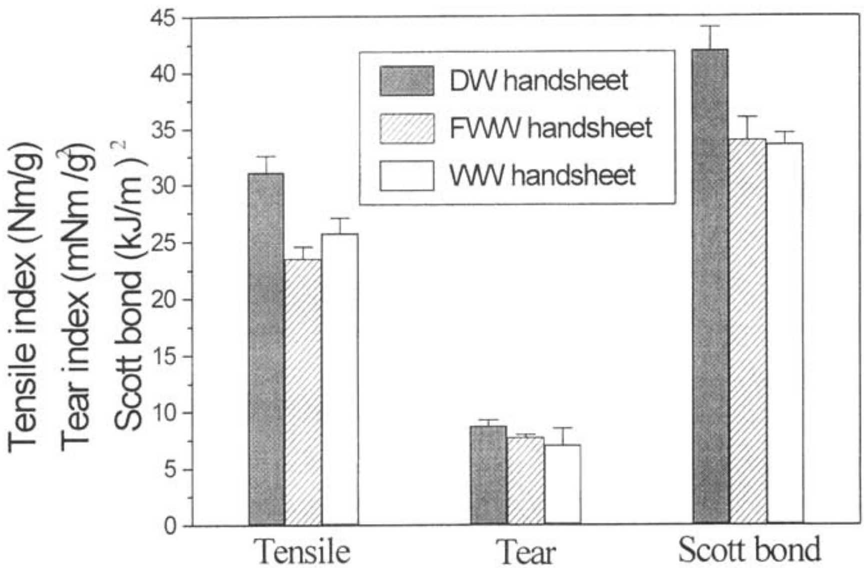

FIGURE 3. The Dry Strength Properties of Handsheets Made Using Distilled Water (DW), Filtered White Water (FWW) and White Water (WW)

from a lower density sheet of the former. In contrast, when handsheets were made from the water containing the colloidal component there was a significant decrease in porosity and rate of water absorption. It can be surmised that the colloidal particles are filling the pores in the paper and consequently, more than counteracting the effects on these properties of the reduced sheet consolidation caused by the dissolved materials. Brightness was not affected by the dissolved components but was reduced by the colloidal organics (Figure 4). This likely results from absorption of light by lignin, which is the main component of the colloidal fraction (Figure 2).

As mentioned earlier, closure of the water systems in the newsprint mill will cause a build-up of both the colloidal and dissolved organic substances

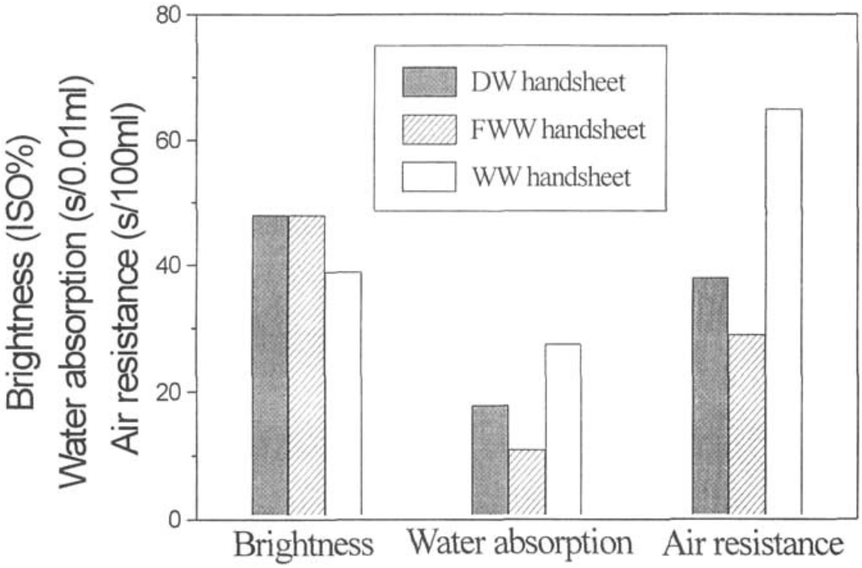

FIGURE 4. The Optical and Absorption Properties of Handsheets Made Using Distilled Water (DW), Filtered White Water (FWW) and White Water (WW)

increasing their impact on paper quality. To maintain paper quality and papermachine runnability the level of these substances in the mill process waters must be controlled.

The Ability of Various Enzymes to Remove Dissolved and Colloidal Substances

We have shown[8] that the fungus *Trametes versicolor* grows well on newsprint mill white water and that both the fungus and its fungal culture filtrate (FCF) are efficient at degrading the dissolved and colloidal substances. The removal of lignans and triglycerides indicated the presence of oxidative enzymes and hydrolases in addition to the expected cellulytic enzymes in the fungal culture

TABLE 2.

Treatment of White Water and Filtered White Water With Laccase

mg/L	WW control	Laccase treated WW	FWW control	Laccase treated FWW
RFA	33 ± 1.2	27 ± 1.4	16 ± 1.4	17.5 ± 1.8
Li & S	171 ± 2.2	2.6 ± 0.3	167 ± 6.2	2.5 ± 0.4
SE	25 ± 0.3	0	18 ± 3.5	0
TG	11 ± 3.5	0	0	0
Lignin	456 ± 5	605 ± 5	215 ± 3.5	390 ± 25
TDCS	2350 ± 125	2300 ± 64	2062 ± 56	2040 ±53

filtrates.[7] Although, using lipase/esterases and cellulase/hemicellulases to treat the process waters of newsprint mills has been well documented,[3-5] little work has been reported so far on using oxidative enzymes to deal with white water DCS problems. To try to obtain a better understanding of the impact of the FCF as well as different groups of enzymes on process waters, we treated the colloid containing (WW) and colloid-free (FWW) white waters with commercial sources of the three main types of enzymes found in the *Trametes versicolor* culture filtrate. The effects of the enzymes on the different classes of dissolved and colloidal substances were assayed and compared to the white water control.

Although, laccase treatment at 45°C for 3 h had little effect on the total amount of dissolved and colloidal substances present in both the WW and FWW fractions, significant changes were observed in the extractives portion (Table 2). For both the WW and FWW, laccase treatment had little effect on the resin and fatty acid component however, there was a marked decrease in the other lipophilic extractives and lignans, and an increase in lignin content. The observation that the decrease in the lignan/sterol portion approximated the increase in the lignin is consistent with laccase causing the polymerization of the low molecular weight

TABLE 3.

Treatment of White Water and Filtered White Water With Lipase

mg/L	WW control	Lipase treated WW	FWW control	Lipase treated FWW
RFA	33 ± 1.2	42 ± 2.2	16 ± 1.4	18 ± 0.4
Li & S	171 ± 2.2	168 ± 2.1	167 ± 6.2	168 ± 0.6
SE	25 ± 0.3	17 ± 0.3	18 ± 3.5	16.6 ± 0.1
TG	11 ± 3.5	0	0	0
TDCS	2350 ± 125	2115 ± 145	2060 ± 56	2020 ± 23

phenolic lignans into a high molecular weight "lignin-like" material. The mechanism involved in the conversion of the extractives after treatment with the laccase is currently under investigation.

As seen in Table 3, treatment with lipase at 45°C for 3 h, had no effect on the dissolved components of the white water (FWW). However in the colloid containing water (WW), it depleted the triglycerides and part of the steryl esters. The reduction in steryl esters corresponded to the amount present in the colloidal form. This was expected as lipases are known to act at colloidal interfaces.[11] The increase in the resin and fatty acid fraction on lipase treatment corresponded approximately to the amount that would be released by hydrolysis of the triglycerides and steryl esters.

Very little of the original neutral carbohydrates in the FWW were present as monomeric sugars (Table 4). The presence of arabinose indicated that a significant amount of highly soluble arabinogalactans were present along with the galactogluconmannans.[12] Treatment of the FWW with cellulases and mannanases resulted in the release of monomeric sugars. During the 3 h treatment at 45°C, cellulase released most of the available glucose, about one third of the arabinose and a small portion of the galactose and mannose. Mannanase treatment was less

TABLE 4.

Sugar Monomers Released after Treatment of Filtered White Water (FWW) with Cellulase and Mannanase

mg/L	Arab.	Gal.	Glu.	Xyl.	Man.
FWW	0	0	0.13	0	0
Cellulase treated	24.1	35.6	124	9.7	58.7.
Mannanase treated	24.6	9.2	61.7	9.6	32.8
Total sugar content	77	428	172	ND	511

effective at hydrolyzing the neutral carbohydrates with even a lower amount of mannose released.

CONCLUSION

The newsprint white waters contained a significant amount of colloidal substances ranging in size from 250 nm to 1000 nm. The lignin and ester-bonded extractives, such as sterol esters and triglycerides were the main constituents of the colloidal particles, while the neutral polysaccharides and lignans were predominantly dissolved in the white water. Reductions in paper strength were mainly caused by the dissolved substances, whereas the colloidal substances are primarily responsible for impairment of paper surface and optical properties. Laccase treatment resulted in the degradation of most of the extractives while the lipase specifically hydrolyzed the ester-bonded extractives present in the colloidal fraction.

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